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Title: IMMUNOGENIC PILI  
 PRESENTING FOREIGN PEPTIDES,  
 THEIR PRODUCTION AND USE  
 Inventor(s): Peter O'Hanley et al.  
 DOCKET NO.: 050939/0104

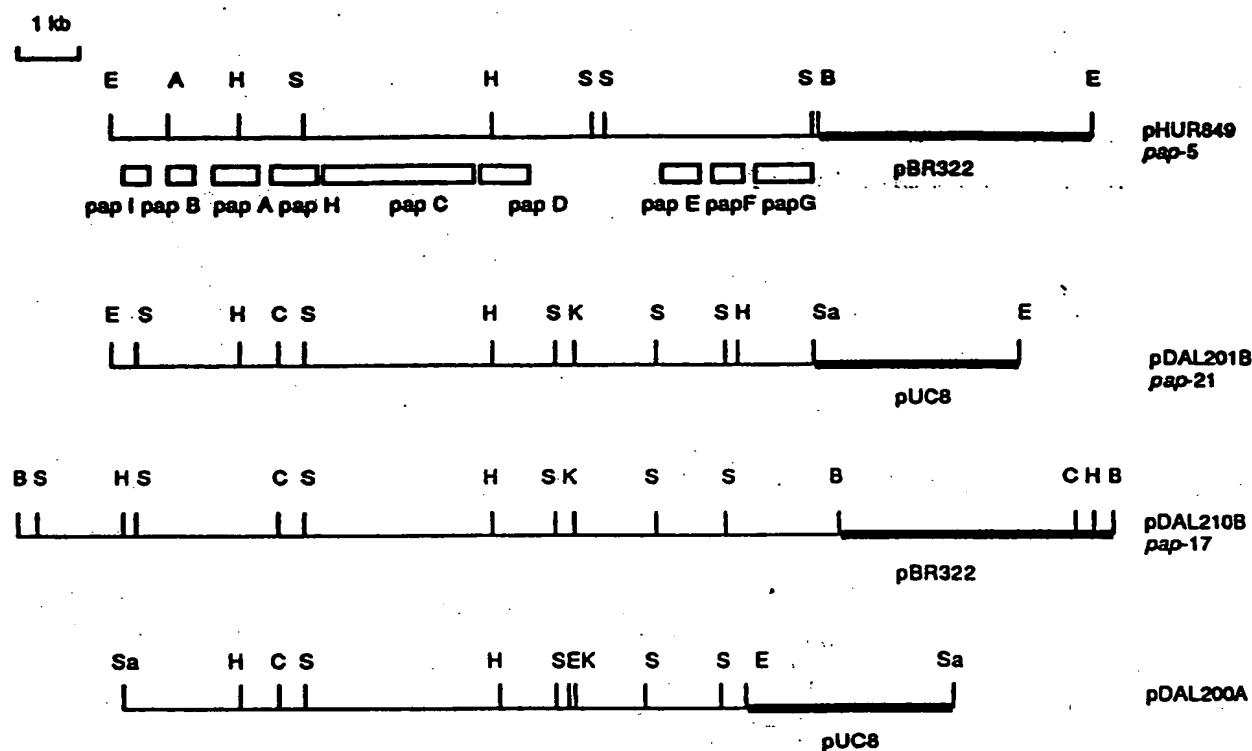


Figure 1. Genetic and physical map of recombinant plasmids pHUR849, pDAL201B, pDAL210B, and pDAL200A. The locations of the *pap* genes shown as open bars. Restriction site abbreviations: A, *Apa* I; B, *Bam* H I; C, *Cla* I; E, *Eco* R I; K, *Kpn* I; Sa, *Sal* I; and S, *Sma* I.

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Figure 2. DNA sequences of pHU8849 (A), pDAL201B (B), pDAL210B (C), and pDAL200A (D) *p45H* structural genes. The nontranscribed DNA strand for each clone is shown. Numbering is from the 5' end. The deduced amino acid sequence for the correct frame is shown below each DNA strand. The first amino acid of the mature protein is +1. Stop codon is marked with asterisks. The restriction sites for *Cla* I (ATGGTA), and *Sma* I (CCGGG), are underlined once, respectively.

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pHUR849	1	ATGAGACTGGATTCTCTGTTCCACTTTCTTTTGGCTGTGTGTTGTCATGGTGT	60
pDAL200A		t	
pDAL201B		t	
pDAL210B		t	
pHUR849	61	TTTGGCCGGTCCGTTCTCCGGCCCGCATGTCCTTCTGAATACCTGGGAGAACAGCAC	120
pDAL200A		t	
pDAL201B		t	
pDAL210B		a	
pHUR849	121	GTATGGTGGACGGCAGGGCTGCTTTCATCGTGAGGTTCTCAGACCTGGCTGTACTCTG	180
pDAL200A			
pDAL201B			
pDAL210B			
pHUR849	181	GGCATGGAAGACCCCTGGCAGATTATTGATATGGGGAAACCCGGTACGGGATTTACAG	240
pDAL200A		c	
pDAL201B		c	
pDAL210B		c	
pHUR849	241	ATTGGTTCTCOGGACCTGAAAGAAAATTCAGCCTCCGGCTCAGGAATTGTGAATTAAAC	300
pDAL200A		c	
pDAL201B		c	
pDAL210B		c	
pHUR849	301	ACTCAGGGTGGGAAACCTTTCTCTGATCCGGATAACGGTGACTTTGATGGCTCCGG	360
pDAL200A			
pDAL201B			
pDAL210B		t	
pHUR849	361	GGTCAAACCCCGATAAGTTAATTATCCGGTCAGGAAAGGCATTAAATCTGAGATA	420
pDAL200A		a	
pDAL201B			
pDAL210B			
pHUR849	421	GCTGATGTCAGGGAAATATTGCCGGCAGGAAAGTAATGCCCTGAAATACCATTTGAGC	480
pDAL200A		c	
pDAL201B		g	
pDAL210B		c	
pHUR849	481	GGTAATGAAAGCGCTGGATTACACCCCTAGAAATTGTGAGAAACGGAAAAACTTGAA	540
pDAL200A		c	
pDAL201B		c	
pDAL210B		c	
pHUR849	541	GGCGGAAATTATTTGCTGCTGGATTCCGGTCCGATTATGAGTGAA	588
pDAL200A		c	
pDAL201B		c	
pDAL210B		c	

Figure. 3. Comparison of deduced nucleotide sequence of *papH* genes pHUR849, pDAL200A, pDAL201B and, pDAL210B. The nucleotide(nt) identities, compared with the deduced sequence of *PapH* nt sequence *papH* gene of pHUR849 (upper case), are indicated by blank space, nt differences for the *PapH* genes of pDAL201B, pDAL210B and, pDAL200A, are shown with the corresponding single letter nt code (lower case), respectively. Numbering is from the 5' end. The first nt of coding for the leader sequence is numbered 1, and the first nt coding for the mature protein is numbered 67.

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-22 -1 +1 18  
 M R L R P S V P L F F F G C V F V H G V P A G P F P P P G M S L P E Y W G E E H  
 C  
 19 58  
 V W W D G R A A F H G E V V R P A C T L A M E D A W Q I I D M G E T P V R D L Q  
 59 98  
 N G F S G P E R K F S L R L R N C E F N S Q G G N L P S D S R I R V T F D G V R  
 99 138  
 G E T P D K F N L S G Q A K G I N L Q I A D V R G N I A R A G K V M P A I P L T  
 a  
 139 173  
 G N E E A L D Y T L R I V R N G K K L E A G N Y F A V L G F R V D Y E

**Figure 4. Comparison of deduced amino acid sequence of *papH* genes pHUR849, pDAL201B, pDAL210B and, pDAL200A.** The vertical arrow indicates the postulated cleavage site for the signal peptidase. The amino acid (aa) identities, compared with the deduced sequence of PapH protein of pHUR849 (upper case), are indicated by blank space, aa differences for the PapH proteins of pDAL201B, pDAL210B and pDAL200A, are shown with the corresponding single letter aa code(lower case), respectively. The first aa of the leader sequence is numbered -22, and the first aa of the mature protein is numbered +1.

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A 1 26 127 173  
GPFPPPGMSLPEYWGEEHVVWDGRAARAGKVMPAIPLTGNEEALDYTLRIVRNGGKLEANGNYFAVLGFRVDOYE  
27 126  
EHGEVVRPACTLAMEDAWQIIGAGKVMPAIPLTGNEEALDYTLRIVRNGGKLEANGNYFAVLGFRVDOYE

B 1 47 127 173  
GPFPPPGMSLPEYWGEEHVVWDGRAAFHGEVVRPACTLAMEDAWQIIGAGKVMPAIPLTGNEEALDYTLRIVRNGGKLEANGNYFAVLGFRVDOYE  
48 126  
DMGETPVRDLONGESPERKESLRBNCCENSOGGNLFSDSRIVTFDDVRGETPDKFNLSGGOAKGINLQIADARGNIA

Figure. 5. Comparison of deduced amino acid sequence of *papH* gene deletion mutants pHUR849-5(*pap-5*), pDAL201B(*pap-21*), pDAL210B(*pap-17*) and, pDAL200A(*pap 200A*). The deduced amino acid sequence of each of the finial constructs is shown, (A) pHUR849-5 and, (B) pDAL201B, pDAL210B and, pDAL200A which are identical to each other. The amino acid identities of the proteins are upper case letters. The first amino acid of the mature fusion protein is numbered 1. The underlined sequence indicates the amino acid residues deleted from the mature fusion protein of each strain.